A DETERMINATION OF THE NUMBERS OF HISTIDIN DECARBOXYLATING ORGANISMS IN THE FECES IN DEMENTIA PRAECOX AS COMPARED WITH THE NUMBERS IN NORMAL FECES

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Several investigations, among which is the work of Berthelot and Bertrand,[1] have shown that an organism possessing remarkable powers for decarboxylating various amino and other organic acids can be isolated by special methods from the intestinal tracts of certain individuals. This organism Berthelot and Bertrand called B. aminophilus intestinalis, and described as similar culturally to B. mucosus, but differing from the latter in this marked property of decarboxylating certain COOH containing acids.

In the case of one of the amino-acids, namely, histidin, the remaining portion of the molecule after decarboxylation is the corresponding amin, histamin. And B. aminophilus, when grown in a histidin-containing medium, produced this intensely toxic histamin in a concentration such that these investigators were able to kill guinea-pigs with doses as small as 0.001 c.c.

Dale and Laidlaw[2] have shown that plain muscle responds with violent contraction when brought in contact with extreme dilutions of histamin. In fact, a method, based on this remarkable physiologic action of histamin, has been developed and is now in common use in testing for the presence of this substance.

With the evidence from these investigations as a starting point, a theory regarding the etiology of dementia praecox has been advanced by Bayard Holmes in recent articles.[3] He believes that the organism discovered by Berthelot and Bertrand, or at any rate, some organism capable of transforming histidin into histamin, is present in the intestinal tracts of dementia praecox patients. Such organisms, by utilizing

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[1] Compt. rend. Acad. d. sc., 1912, 154, p. 1826.

[2] Jour. Physiol., 1910, 41, p. 318.

[3] Chicago Med. Recorder, 1916, 38, p. 60. Am. Med., 1916, 22, p. 405.

the histidin set free in the intestinal tract by digestion of protein, could then produce the intensely toxic amin, histamin. The histamin would be absorbed by the blood, and then, because of this powerful physiologic action on plain muscle tissue, would cause a constriction of the ring of Cannon. This constriction would bring about a condition of cecal stasis, which by holding the food residues in the cecum for several days, would favor the development of more histamin-forming organisms, with more formation and absorption of the toxic base, and further construction of the ring of Cannon—the various phenomena forming a vicious cycle, with constant poisoning of the system with histamin. This prolonged poisoning of the various organs of the body might show itself in various ways, but its action on the nervous system is assumed to be more or less specific and to give rise to the symptoms seen in dementia praecox.

In a recent article,[4] the author of this theory describes how the condition of cecal stasis, lasting from 4-5 days in dementia praecox, has been demonstrated as a fact, by fluoroscopic methods. Furthermore, he asserts that histamin has been isolated by chemical methods, and that bacteria capable of producing histamin from histadin are present in the intestinal tracts of such patients.

The investigation reported in this article was undertaken to determine the significance of this latter statement, namely, to compare the relative numbers of histamin forming organisms in a given weight of stool from dementia praecox patients with the relative numbers of similar organisms from a similar weight of stool from normal subjects.

TECHNIC

At the outset it was considered necessary to isolate the suspected variety or varieties of organisms which, when grown in pure culture in a histidin containing medium, can produce a detectable quantity of histamin, and to establish some of the biochemical characteristics by which such organisms could be distinguished from other associated varieties. The method of enrichment, used by Berthelot and Bertrand, was used, and after an examination of the stools of some dozen or more patients and controls, an organism was finally isolated which readily converted histidin into histamin. It was found to be similar to the organism described by Berthelot and Bertrand. A loopful of feces was inoculated into the histidin containing medium and after enrichment in two successive transfers in this medium, the organism, when streaked on Endo plates, appeared as a rapidly growing, white, semi-translucent, mucuslike colony. It is a capsulated, nonspore bearing, gram-negative, nonmotile, facultative anaerobic bacillus, producing on agar slants a mucuslike growth similar to B. mucosus.

[4] Med. Council, 1917, 22, p. 31.

It was found later, however, that the latter may be distinguished from this histamin forming variety when grown in glycerol broth. The histamin forming variety is unable to ferment glyecrol.

Incidentally, this organism was isolated, not from one of the patients, but from a supposedly normal control, and in spite of the fact that the enrichment method was used in the search for it, it did not appear in any of the other stools examined.

It was then thought possible that by devising some selective plating medium on which colonies of this organism could be recognized directly, it would be a simple matter to determine the relative numbers of the organism in each sample of stool when plated directly on such medium. Accordingly a medium, prepared after the following method, was used for this purpose: 0.1 gm. of histidin-hydrochlorid, 0.025 gm. KNO_3, 0.002 gm. $CaCl_2$, and 0.02 gm. K_2SO_4 are dissolved in a small flask in about 50 c.c. of distilled water; 0.02 gm. $MgSO_4$ dissolved in 10 c.c. of water is added to this; 0.5 gm. K_2HPO_4 dissolved in water is then added, and the entire amount made up to 100 c.c.; 0.8 gm. agar is then washed and added to the solution, and the mixture sterilized at 15 lbs. for 15 minutes. The agar is dissolved by this process, and when mixing the medium, care should be taken not to form air-bubbles in the mixture. Now add 1.2 c.c. of a 10% solution of Na_2SO_3 and titrate the solution by running in N/10 HCl, 1 c.c. at a time, until the medium is neutral to litmus paper. Three drops of a saturated alcoholic solution of basic fuchsin are now added and the medium after being thoroughly mixed is poured into plates. The precipitate of $Ca_3(PO_4)_2$ will not interfere with the purpose for which the medium is to be used and should not be filtered off, since even the slight solubility of this solid is sufficient, as well as necessary, for the growth of this organism.

On such a medium the organisms of the intestinal tract were soon found to divide themselves readily into 5 different groups: (1) those which are unable to develop in the presence of the basic fuchsin, and which are promptly eliminated from the group to be studied; (2) those which, on a medium containing such elementary substances, are unable to produce visible colonies; (3) those which are able to produce only very minute colonies; (4) those which grow readily, but which, even in the absence of sugars, are able to restore the color of the fuchsin and thus appear as red colonies, and (5) equally rapidly growing colonies which do not become red, but which show varying degrees of translucence.

In the case of the organism in question, which shall be referred to as B. aminophilus, a peculiar characteristic was revealed which was not duplicated by any other variety encountered. It appeared after 24 hours at 37 C. as a colony of from 2-3 mm. in diameter, having the appearance of a clear colorless drop of water. This remarkable translucence, which even B. mucosus could not imitate, was retained indefinitely, so long as the agar was kept from drying up.

To determine the efficiency of this medium in disclosing the presence of B. aminophilus in a given sample of stool the following experiment was made:

Two grams of an average sample of stool from a patient suffering from catatonia and other pronounced symptoms of dementia praecox, were thoroughly mixed with a mortar and pestle. One gram was then removed to a watch glass, while the remaining portion in the mortar was inoculated with 2 loopfuls of B. aminophilus, and mixed again. This portion was then removed to a 2nd watch glass. One half of each portion was again removed to separate mortars and diluted and mixed with 5 c.c. of water. Dilutions 1:100,000 were now made of each sample, and plated on 10 plates of the histidin agar described, by placing 1 drop of the dilution in the center of the plate and spreading it over the agar surface with a sterile bent glass rod. After 48 hours at 37 C. each of the 10 plates inoculated with the sample to which had been added the 2 loopfuls of the B. aminophilus culture, showed a count of approximately 150 colonies per plate. About 10% of these were of the B. aminophilus type, which appeared as clear colorless watery colonies, and which were subsequently identified as B. aminophilus. The other 10 plates, inoculated with the sample which had not been mixed with the B. aminophilus culture, showed about the same number of colonies, but none of those with the watery appearance of the B. aminophilus type could be found on any of the 10 plates. Furthermore, plain agar plates, inoculated at the same time with the same dilution of this sample, revealed the fact that this selective medium permitted only about one twentieth of the intestinal flora to develop visible colonies. In other words, out of 10 plates, representing 150 bacteria each, or 1,500 in all, not one colony of B. aminophilus could be found, and if these 1,500 colonies represent only one-twentieth of the living bacteria of the intestinal flora, it means that if B. aminophilus was present in this sample of stool, it was present in a ratio of less than 1:30,000 of other living bacteria in the sample. Obviously, for practical purposes, the organism may be considered absent. The stools from 6 other patients were then examined by the same method, and the same negative results obtained in every case.

In order to determine if the absence of the organism may have been due to the fact that, although present in the upper portion of the intestinal tract, it might have perished later in the lower portion of the colon, the following tests were made: The 2 samples of 0.5 gm. each remaining on the watch glasses, were held anaerobically at 37 C. for 48 hours, and the process of plating then repeated. It was found that, although under conditions approximating those existing in the colon, and which presumably might be unfavorable for the organism, B. aminophilus is not destroyed, but on the other hand, increases out of proportion to the rate of increase of the other histidin utilizing varieties.

Throughout the investigation, however, a continual search was kept up for other possible varieties which could produce histamin from histidin, and for this purpose the medium was modified by leaving out the Na_2SO_3 and fuchsin, since either of these two substances might suppress the growth of some histamin forming varieties. Furthermore, plates incubated over periods of two weeks, some under aerobic, and

others under anaerobic conditions, revealed no other histamin forming variety.

The special enrichment method used by Berthelot and Bertrand, and Mellanby and Twort,[5] are obviously unsuited for determining the relative number of a given organism in mixed culture, since a single living individual of the given organism, among millions of other bacteria, might eventually gain the ascendency in the enrichment medium and then be easily isolated. The reason for this is probably the following: The medium used by Berthelot and Bertrand, for example, when inoculated with such a heterogeneous mixture of varieties as is found in feces, rapidly becomes alkaline. This alkalinity becomes the limiting factor in the multiplication of the different varieties, and quite naturally, those varieties possessing the greatest tolerance for this alkalinity will be permitted to further increase their numbers long after the other varieties have been overcome by the alkaline conditions of the culture. Given then, an organism possessing a high rate of multiplication plus a high degree of tolerance for hydroxyl-ions, such an organism, if present, will naturally gain the ascendency in a few transfers in such a medium. In this case, B. aminophilus is such an organism. It has a rate of growth perhaps not exceeded by any other organism commonly found in feces, and in addition is able to continue its metabolism in an alkalinity represented by a P_H^+ of 8.7. With these two characteristics in its favor, it should be able to compete with the other varieties in the mixed culture, and to gain the ascendency after a few transfers in this medium. Then by plating the growth obtained in the last transfer, B. aminophilus should be easily isolated. Such a method, used, as previously stated, by Berthelot and Bertrand, was attempted a second time. One set of transfers was held under aerobic, and another under anaerobic conditions, and finally streaked on histidin agar plates. In most cases the method resulted in yielding a pure culture of some type, from each stool examined, usually of the lactis aerogenes variety. Occasionally, B. mucosus variety was found and in one case a rapidly growing, vigorous liquefier of gelatin was obtained. B. cloacae was isolated once. In no case, however, out of 38 different samples of stools taken from dementia praecox patients and normal controls, was the B. aminophilus or any other histamin forming variety discovered. The method used for detecting histamin formation in these cultures was the method devised by Dale and Laidlaw,[2] and

[5] Jour. Physiol., 1912, 45, p. 53.

claimed by these and other investigators to be delicate to 1 part of histamin in 250,000,000 parts of water. Some other method, however, may possibly lead to more successful results in demonstrating the presence of histamin forming varieties in the stools in dementia praecox.

But even granting that such organisms are present in these patients it would still remain to be shown what significance their presence may have, by showing that the same organism is not present in the intestinal tracts of normal subjects, which so far as the literature reveals, has not been done. The failure to find appreciable numbers of histamin forming varieties in the stools in dementia praecox, however, does not prove that histamin should not be present in such stools. It is commonly known that two different varieties of organisms, neither of which in pure culture can produce a given chemical change, when grown in mixed culture may produce that change. On the other hand, their presence, even in large numbers, would not justify the assumption that histamin should also be found there, as was shown most clearly by the following experiment:

One hundred c.c. of the histidin containing medium used by Berthelot and Bertrand was inoculated with B. aminophilus, and incubated at 37 C. for 3 days. The culture was then killed by boiling, neutralized with N/10 HCl and divided into 3 parts. Part 1 was inoculated with feces from a praecox patient; Part 2 with B. aminophilus, and Part 3 was left uninoculated. All 3 parts were held at 37 C. for 3 days, and then tested for the presence of histamin. Part 1 gave a negative, Part 3 a positive, and Part 2 a still stronger positive reaction for histamin.

These results show 3 things: First, that histamin formed by B. aminophilus can be destroyed by other intestinal bacteria; second, that B. aminophilus probably can use the histidine carboxyl (COOH) group only, and third, that the alkalinity of the culture becomes the limiting factor in the production of histamin, by the bacterial method, and that with successive neutralizations of the culture, a larger yield of this valuable product should be obtained. (Regarding the latter point, an investigation of the value of such a method is now being made.)

From the work of Kendall[6] and others, it was predicted that when B. aminophilus is supplied with other available sources of carbon, for example, dextrose, it would not use the carboxyl (COOH) portion of histidin, and therefore would not form the amin. Dextrose was added

[6] Jour. Med. Research, 1911, 25, p. 117.

to the histidin containing medium and after incubating with B. aminophilus for three days, the culture was examined for the presence of histamin. A negative reaction was obtained, thus verifying the prediction. The same result was obtained when tyrosin was used in place of histidin. This same experiment was done by Mellanby and Twort,[7] but these investigators interpreted the results to mean that histamin formation was prevented because the acid reaction, resulting from the fermentation of the carbohydrate, was unfavorable for the decarboxylating action of the bacteria. They came to this conclusion because a tube of medium, rendered acid purposely, at the time of inoculation, also failed to yield histamin. Unfortunately, they do not state how much acid was used, although they admit that growth in a sugar free medium resulted in a final alkaline reaction. As a matter of fact, B. aminophilus when grown in a medium purposely made acid to a reaction of as high a P_H^+ as 5.2, will promptly turn the medium alkaline, and histamin formation proceeds normally. Higher concentrations of hydrogen-ion do prevent histamin formation, but only because B. aminophilus then fails to grow. In general, it may be said that B. aminophilus does not form the amin through choice, but only by dint of the circumstances imposed on it, namely, by being forced to subsist on a medium containing only the one source of utilizable carbon, histidin.

It is noteworthy that histamin formation goes on in a medium showing a marked alkalinity, in a range of P_H^+ inimical to the activities of most intestinal bacteria. Under this condition, the histamin formed in the intestine could accumulate, since the other varieties, which at higher ranges of P_H^+ would promptly demolish the histamin molecule, are now inactive. It is also noteworthy that stools from persons suffering from constipation invariably show a marked alkalinity, a P_H^+ as low as 8.6 in some cases observed. In such a stool, provided other sources of utilizable carbon are absent, formation of the amins (not necessarily histamin only) could proceed, and these, if not absorbed, could accumulate. But that histamin can be isolated by chemical means from the stools of dementia praecox patients, as stated by the author of this theory, has no significance in establishing the validity of the theory until it is also proved that the same substance is not present in the highly alkaline stools of other people. As a matter of fact, histamin has been shown to be a normal constituent of the intestinal

[7] Jour. Physiol., 1912, 45, p. 57.

mucosa. The works of Bayliss and Starling,[8] and Barger and Dale[9] are significant in this connection. Their investigations, undertaken separately and for different purposes, proved that histamin is easily extracted from fresh intestinal mucosa. They make no attempt to explain its presence here, but simply point to the fact that histamin is a constant constituent of the lining of the intestine. Furthermore, during this investigation, an examination of a water extract of a stool from one of the control subjects gave a strong Pauly reaction, and an oxytocic reaction for tyramin (which is formed from tyrosin by the same organism forming histamin from histidin).

With regard to the cecal stasis said to occur in dementia praecox by the author of this theory, it may be said that in 4 cases of dementia praecox examined by me, and 3 other cases of which roentgenograms had been made, no indications of a cecal stasis such as would result from a constriction of the ring of Cannon was seen, and certainly nothing like a cecal stasis of from 4-5 days' duration. The most that could be said of these cases was that in two of them, the roentgenograms revealed a retention of the barium meal, not in the cecum, but in the pelvic colon, for a period somewhat longer than normal; and even this condition, which was nothing more than the ordinary constipation commonly seen in dementia praecox, was only temporary.

SUMMARY

An organism similar to the one described by Berthelot and Bertrand has been isolated; it is shown to differ from B. mucosus by its inability to form gas and acid in glycerol broth.

This organism, called B. aminophilus, does not form histamin from histidin when more available source of carbon of the carbohydrates is present.

A selective plating medium for isolating this organism from mixed culture has been devised. By this medium it was shown from an examination of samples from 38 different stools, that B. aminophilus ordinarily is not found in the human intestinal tract in sufficient numbers to be isolated by direct plating of a quantity of feces representing from 20,000 to 100,000 living bacteria.

[8] Jour. Physiol., 1902, 28, p. 335.
[9] Ibid., 1911, 45, p. 499.